REMARKS

This Supplemental Amendment supplements the Amendment filed on December 6, 2007, to fix typographical translational errors in claims 20 and 28 and in the Remarks (page 10, line 17). Additionally, three additional sentences are added to the Remarks on page 10, after line 18. The following Remarks are repeated in this Supplemental Amendment and the changes are shown in bold type for convenience of the Examiner to show the differences in the Remarks from the ones made in the Amendment filed on December 6, 2007.

The present Amendment amends claims 20 and 28.

No new matter has been added.

Specification

In response to the objections to the Specification under 35 U.S.C. § 132(a) on Page 3, Paragraph 6. of the Office Action, Applicants again respectfully assert that the Amendments to the Specification are appropriate and correct. Applicants would like to emphasized that these errors are clearly mistakes that were caused during translation of the Japanese-language application into English.

Under MPEP 2163.07, Amendments to Application Which Are Supported in the Original Description, II. Obvious Errors, in column 2, first full paragraph, it states

> Where a U.S. application as originally filed was in a non-English-language and an English translation thereof was subsequently submitted pursuant to 37 CFR 1.52(d), if there is an error in the English translation, applicant may rely on the disclosure of the originally filed non-English-language U.S. application to support correction of an error in the English translation document.

In response to the Notification of Missing Requirements, Applicants submitted an English Language Specification (along with other application documents) as required under 37

CFR 1.52(d) on June 1, 2005. In accordance with MPEP 2163.07, Applicants have properly corrected errors in the English language translation.

Under MPEP 201.13, Section II-G., on page 200-79, second paragraph, it states:

> For US applications filed on or after September 21, 2004, a claim under 37 CFR 1.55 for priority of a prior-filed foreign application that was present on the filing date of the US application is considered an incorporation by reference of the prior-filed foreign priority application as to inadvertently omitted material....

The present application was filed in the United States on June 1, 2005, and therefore meets the requirement under MPEP 201.13, Section II-G. The inadvertently omitted material is the accurate subject matter disclosed in the priority document.

The Amendments to the Specification do not add new matter, but, as previously stated, were made merely to correct obvious errors in the English translation of the text based on the original Japanese text (JP 2002-167920).

Withdrawal of the objection is respectfully requested.

Claim Rejections- 35 U.S.C. § 103

In the Action, claims 20 and 28 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Kawahara et al., *Quantitative analysis of protein synthesis altered by estrogen in cultured Xenopus liver parenchymal cell*, Develop., Growth and Differ. 23, 599-611 (1981) ("Kawahara") in view of Dunbar et al., *Preparation of Polyclonal Antibodies*, Methods in Enzymology 182, 663-670, (1990) ("Dunbar") and Harlow & Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 283, 285-293, and 331 (1988) ("Harlow"). Claims 20 and 28 were further rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Shapiro et al., *In Vitro Translation and Estradiol-17β Induction of Xenopus laevis Vitellogenin Messenger RNA*, The Journal of Biological Chemistry, Vol. 251, No. 10, pp. 310-3111, (1976) ("Shapiro") in view of Kawahara and Harlow & Lane

("Antibodies: A Laboratory Manual" (1988) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pages 283, 285-295, 302-303 and 313). The rejections are respectfully traversed.

The feature of the claimed invention lies in performing (1) the absorption purification using male blood serum and (2) the affinity-purification using an antigen (vitellogenin) column after the absorption purification.

Although Kawahara includes statement (1) above, there is no suggestion or disclosure regarding statement (2) above. Harlow includes a description on the generalized purification method including (2) the affinity-purification. However, there is no technical disclosure or suggestion from a viewpoint of purifying antibodies having a high antibody titer and specificity for utilizing vitellogenin as a biomarker. Note that Dunbar relates to the isolation of the IgG fractions, and therefore is considered not corresponding to the cited reference in this case.

As has been indicated by the empirical data filed previously, specificity of antibody obtained by the affinity purification after the absorption purification (antibody 1) was 30-fold higher than that of antibody by the absorption purification after the affinity purification (antibody 2). The fact that there has been no report up to this time on the generation of this much difference between antibody 1 and antibody 2 proves that even those skilled in the art did not expect this much effect. Further, the antibody which enables evaluation of chemical substances/environment with vitellogenin as the biomarker was first obtained by the purification method in the order of (1) to (2) (a kit for attaining the high sensitivity 0.2 mg/ml detection level was also obtained since ELISA is structured with the antibody obtained by this method).

According to the method of the present invention, it is possible to purify a large number of antibodies having a certain high level of quality.

Furthermore, Applicants believe that portions of the content of the outstanding Office Action are incorrect, to wit:

Regarding the last paragraph on page 13, the document of Kawahara et. al. includes the description that VTG specific antibody can be obtained by the absorption purification. However, the technique is actually different from that of the present invention. Therefore, the antibody titer and the degree of specificity of the antibody that can be obtained differ. The Examiner

describes that, regarding the fact that Kawahara et. al. includes no description on the column, it is the same as that by the "batch" method. However, the "batch" method in the cited Harlow & Lane is a method using DEAE-MATRIX, which is totally different from the method of Kawahara et. al in which the blood serum is used as it is. The "batch" method using DEAE-MATRIX in Harlow & Lane is, in the first place, described as a method for removing proteins other than the antibody from things like anti-blood serums and is not an absorption purification method for raising specificity of the antibody. Therefore, it cannot be said that "purification steps need not include a column in order to achieve separation or purification, but rather can be performed in "batch"".

If DEAE-MATRIX is used, the used proteins can be removed together with the DEAE-MATRIX after absorption purification. However, those cannot be removed by the method of Kawahara et. al.

Regarding lines 4 to 5 on page 14, the Examiner indicates that the effect of selecting the antibody that binds to VTG in the absorption purification is the same regardless of whether the column is used or not, which is incorrect. The absorption purification bears an effect of removing the antibodies that bind to the male blood serum proteins except VTG rather than selecting the antibodies that bind to the VTG. Therefore, although Kawahara et. al. includes the description "vitellogenin-specific antisera", because a large amount of male blood serum proteins (including IgG of a frog) remain in this antisera, it can easily be imagined that it is not **pure** vitellogenin-specific antisera. **In other words, Kawaharas' antiserum is vitellogenin-specific, but impure. Even if IgG should be purified from this antiserum by DEAE-Sephadex chromatography or other available methods, the resulting IgG fraction would be contaminated with the frog serum IgG. This impurity severely reduce performance (sensitivity) of the ELISA. Furthermore, even if the antiserum is directly applied on the VTG-affinity column, the column will bind not only anti-VTG antibodies but also other positively-charged proteins that are present in the frog serum due to the acidic nature of VTG-bound column.**

Regarding the last paragraph of page 8, the Examiner indicates that no structural difference in polyclonal antibody that can be obtained is caused between female-derived vitellogenin and

male-derived vitellogenin as immunizing antigens. However, when proteins unique to the female is mixed in the vitellogenin solution purified from female blood serum, antibodies corresponding thereto are contained in the obtained antisera, whereby the antibodies cannot be removed by the absorption purification using the male blood serum. In other words, for effectively performing the absorption purification using male blood serum, it is necessary to immunize the vitellogenin solution induced into the male blood serum. As a result, however, it may be for the structure of the polyclonal antibody against vitellogenin, a difference is caused in the quality of composition when focusing on the composition of the whole polyclonal antibody. In actuality, as a result of raising the specificity by completely removing the cross-reactivity to the male blood serum proteins, an increase in sensitivity was realized in the sandwich ELISA method not by the monoclonal antibody but by the polyclonal antibody of the same lot.

It is respectfully submitted that that none of the applied art, alone or in combination, teaches or suggests the features of the claims as discussed above. Thus, it is respectfully submitted that one of ordinary skill in the art could not combine the features of the applied art to arrive at the claimed invention because the applied art is devoid of all the features of the claimed invention. As a result, it is respectfully submitted that claims 20 and 28 are allowable over the applied art.

Withdrawal of the rejection is respectfully requested.

Furthermore, Applicants respectfully request that the Examiner clarify where the motivation of obtaining an antibody high in specificity of the method of the present invention is disclosed in the cited document.

CONCLUSION

In view of the above amendment, Applicants believe the pending application is in condition for allowance.

Applicants believe no fee is due with this response. However, if a fee is due, please charge our Deposit Account No. 18-0013, under Order No. OMY-0041 from which the undersigned is authorized to draw.

Dated: December 18, 2007

Respectfully submitted,

By _____

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Enclosure(s): Amendment Transmittal

DC298948.DOC